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Improvement of small intestinal microcirculation by postconditioning after lower limb ischemia

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ABSTRACT

Background: Major lower limb vascular surgeries may result in severe, remote injury of the gastrointestinal system, which has high mortality rates. Postconditioning is a technique with potential capability of reducing remote gastrointestinal complications. Our aim was to assess the remote macro- and micro-hemodynamic changes of the small intestine following an infrarenal aortic occlusion and to evaluate the effects of postconditioning on these alterations.

Methods: Rats underwent 3 h of infrarenal aortic occlusion followed by 4 h of reperfusion. In one group, postconditioning was applied. Blood pressure, superior mesenteric artery flow and mucosal microcirculation of the duodenum, jejunum and ileum were assessed. Samples were taken from each intestinal segment for histological examinations.

Results: Superior mesenteric artery flow, as well as microcirculation of the duodenum, jejunum and ileum showed significant impairment in the IR group, while histological damage was significantly worsened. Postconditioning was able to limit flow reduction in all three small bowel segments and in the superior mesenteric artery, and was able to significantly reduce histological damage. Strong negative correlation was found between microcirculatory values and histological damage.

Conclusions: Microcirculatory impairment might be responsible for remote intestinal injury following infrarenal aortic occlusion. Postconditioning was able to reduce this remote intestinal damage.

Introduction

Ischemic-reperfusion (IR) injury of the lower limb is a frequent entity in the field of vascular surgery. Major vascular operations necessitate the exclusion of even the infrarenal section of the aorta which could result in significant ischemic muscle damage (Albani et al., 2000). Lower limb ischemic-reperfusion injuries however carry the possibility of the development of more serious complications such as systemic inflammation and multiple organ dysfunction (Norwood et al., 2004).

Injury of the gastrointestinal tract can be a serious consequence of major vascular operations involving large muscle masses. Previous animal and human studies demonstrated that lower limb ischemic-reperfusion injuries could lead to intestinal mucosal damage (Leng et al., 2011; Weeks et al., 2007; Yassin et al., 1996, 1997; Zhang et al., 2011), increased intestinal permeability (Corson et al., 1992; Edrees et al., 2003; Harkin et al., 2001, 2007; Yassin et al., 1997), endotoxemia (Cabie et al., 1993; Corson et al., 1992; Foulds et al., 1997; Lau et al., 2000; Yassin et al., 1996, 1998), as well as bacterial translocation (Willoughby et al., 1996; Yassin et al., 1998). The increased intestinal permeability facilitates the entrance of endotoxin and gut flora into the circulation which aggravates the already existing systemic inflammation therefore possibly increasing mortality rates (Doig et al., 1998; Keel and Trentz, 2005). However, there is no evidence available on the underlying pathomechanisms of the known phenomenon. It has been hypothesized that remote gut damage might develop as a consequence of systemic inflammation following revascularization. Furthermore some authors suggest that the injury of the gastrointestinal tract might be caused by vascular disturbances (like consequent splanchnic ischemia) (Willoughby et al., 1996), although it was not assessed before in detail. Although it has been demonstrated that the distal part of the colon suffers some degree of ischemic insult during abdominal aortic surgery (Soong et al., 1994) – which is most likely caused by the unavoidable exclusion of the inferior mesenteric artery (Senekowitsch et al., 2006) – small intestinal damage might be also present after a lower limb ischemic scenario. However the small intestinal damage cannot be explained by the unavoidable occlusion of the mesenteric vessels alone, since previous studies indicated that the

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external strangulation of the extremity could also induce injuries in the small intestine (Harkin et al., 2001, 2007), in which case the mesenteric vessels are definitely not occluded. No studies were conducted so far to reveal the underlying cause for this phenomenon.

Nevertheless unfortunately very limited therapeutic options are available for the treatment of serious, life threatening complications of lower limb ischemia (such as intestinal damage) aside from supportive intensive therapy (Gillani et al., 2012), therefore the prevention of IR injuries has got into the focus in the recent years. Postconditioning (PC) is a surgical technique, consisting of brief reperfusion and re-occlusion cycles at the very onset of reperfusion, which were reported to be capable of reducing IR-injury of the lower limbs (Charles et al., 2011; Guo et al., 2011; Liang et al., 2013; Mansour et al., 2012; Sinay et al., 2008). Our previous results showed that postconditioning was able to reduce damages in remote organs such as the lungs and kidneys following lower limb ischemia (Gyurkovics et al., 2011), and it has also been demonstrated that postconditioning is able to limit remote small intestinal mucosal damage (Leng et al., 2011). Nevertheless the remote hemodynamic effects of postconditioning on mucosal microcirculation were not addressed before.

Therefore we aimed to investigate the importance of circulatory disturbances in the development of remote small intestinal injury after a lower extremity ischemia. For this macro- and microhemodynamic changes of the small intestine were evaluated in a rat model of an infrarenal aortic occlusion. Our further objective was to test the favorable effects of the promising technique of postconditioning on these mesenteric and microcirculatory alterations.

Materials and methods

Male Wistar rats weighing 200–250 g (n = 72) were used (Charles Rivers Hungary Ltd, Budapest, Hungary). The experiment was designed in accordance to the National Institutes of Health guidelines on the use of experimental animals and was approved by the Committee on Animal Experimentation of Semmelweis University.

Experimental design

The animals were kept under standard laboratory conditions in 12-hour day–night cycles at 22–24 °C and had free access to commercial pellets and water. Twelve hours prior to the operation only water was provided. Each experiment was started at the same time of the day to avoid the effects of circadian rhythm.

Rats were randomly distributed into 3 groups according to the operation implemented (sham-operation, IR and PC, n = 6/group).

Animals were anesthetized with intra-peritoneal injection of ketamine (75 mg/bw kg) and xylazine (7.5 mg/bw kg). Deep anesthesia was maintained by the intravenous administration of anesthetics (ketamine and xylazine; 25 and 2.5 mg/bw kg/h respectively) and saline solution (3 ml/bw kg/h) via a 22-gauge polyethylene catheter (Harvard Apparatus, Holliston, MA) placed into the right jugular vein. Another polyethylene catheter was inserted into the carotid artery in animals subjected to macro-hemodynamic measurements (n = 18; 6/group) to monitor mean arterial blood pressure (MAP). Body temperature was maintained between 36.5 and 37.5 °C throughout the experiment by a heating pad connected to a rectal thermometer (Homoeothermic Blanket Control Unit, Harvard Apparatus).

Through a median laparotomy the infrarenal section of the abdominal aorta was mobilized. In animals subjected to macro-hemodynamic measurements an ultrasonic flow probe (T206 Animal Research Flowmeter; Transonic Systems Inc., Ithaca, NY) was placed around the superior mesenteric artery to assess blood flow. In separate animals subjected to microcirculatory measurements the middle part of anti-mesenteric surface of the duodenum (n = 18), jejunum (n = 18) and ileum (n = 18) was opened longitudinally through a 10 mm long incision and a laser Doppler flow probe (DP1T surface probe, Moor Instruments Ltd, London, UK) was placed on the mucosal surface to evaluate microcirculation.

After a 30 minute recovery period, occlusion was applied on the infrarenal aorta with the use of an atraumatic microvascular clip (Aesculap YASARGIL FT260T; B. Braun Melsungen AG, Melsungen, Germany). The abdominal cavity was covered with a plastic wrap to prevent fluid loss by evaporation. Three hours of bilateral lower limb ischemia was established. At the end of ischemia the microvascular clip was removed and 4 h of reperfusion was allowed. In one part of the animal population postconditioning (10 second reperfusion, 10 second re-occlusion in 6 cycles) was applied at the onset of reperfusion. Sham-operated animals underwent the same procedure described above except the aortic occlusion. At the end of reperfusion, animals were euthanized with administration of lethal dose of anesthetics then the entire small intestine was carefully removed and divided into the duodenum, jejunum and ileum. A 2 cm long section was harvested from the middle portion of the corresponding bowel segment for histologic examination.

Macro-hemodynamic measurements

Pressure signals (BPR-02 transducer; Experimetria Ltd, Budapest, Hungary) and superior mesenteric artery blood flow (T206 Flowmeter; Transonic) were measured continuously. Hemodynamic data were registered with a computerized data-acquisition system (SPEL Advanced Haemosys 2.46, Experimetria).

Microcirculation

Mucosal microcirculation of the four bowel sections was measured by laser Doppler flowmeter (Moor DRT4, 2 mW laser power at λ = 632.8 nm, Moor). Microcirculatory flow was measured continuously. Data were registered with the manufacturer's own software (MoorSoft for Windows v1.2, Moor). To eliminate the variations among the flow of the individual animals the results were normalized against the baseline flow and expressed as a percentage (Rosero et al., 2011).

Histology

The harvested bowel samples were fixed in 4% neutral-buffered formalin for a day then dehydrated and embedded in paraffin. Thereafter 3 μm thin cross sections were cut and stained for hematoxylin and eosin. Histological examinations were carried out with light microscopy (Olympus BX50 microscope equipped with Olympus DP70 camera; Olympus Corporation, Tokyo, Japan). The histopathological changes of the small intestine were evaluated with the use of a semiquantitative score described by Chiu et al. (1970), which is utilized to quantify the degree of mucosal injury on a 1 to 5 scale. The examining pathologist was not informed about the applied treatment.

Statistical analysis

All values are expressed as means ± s.e.m. The assumption of normality was assessed with the Shapiro–Wilk test. Accordingly, two-way analysis of variance (ANOVA) with repeated measures was used for comparison of all groups with Bonferroni's post-hoc analysis regarding macro-hemodynamic and microcirculatory measurements. Kruskal–Wallis H non-parametric test was conducted for data comparisons in case of semiquantitative histology. Spearman's method was used to assess data correlation between microcirculation and histological score. A 95% confidence interval was considered as statistically significant ($P < 0.05$). Statistical calculations were performed using IBM SPSS Statistics 20.0 software (IBM Corporation, Armonk, NY).

Results

Blood pressure analysis

Blood pressure parameters did not differ significantly in the sham operated group. MAP elevated significantly after aortic occlusion both in the IR ($P = 0.003$) and PC ($P = 0.025$) groups compared to the sham operated group, then remained elevated throughout the period of ischemia in both groups. At the onset of reperfusion there was a temporary, but significant drop in MAP in both IR ($P = 0.009$) and PC ($P = 0.049$) groups compared to the ischemic values. MAP did not differ significantly from the sham operated group in any measured reperfusion time points. No significant disparity was detectable in blood pressure between the IR and the PC groups at any measured time points (Fig. 1A).

Superior mesenteric artery blood flow registration

SMA blood flow significantly impaired by approximately 60% during reperfusion in the IR group compared to the sham operated group ($P < 0.05$). PC was able to significantly improve SMA flow during reperfusion at every measured time point compared to the IR group ($P < 0.05$). No statistically significant difference could be observed between the PC and the sham operated groups (Fig. 1B).

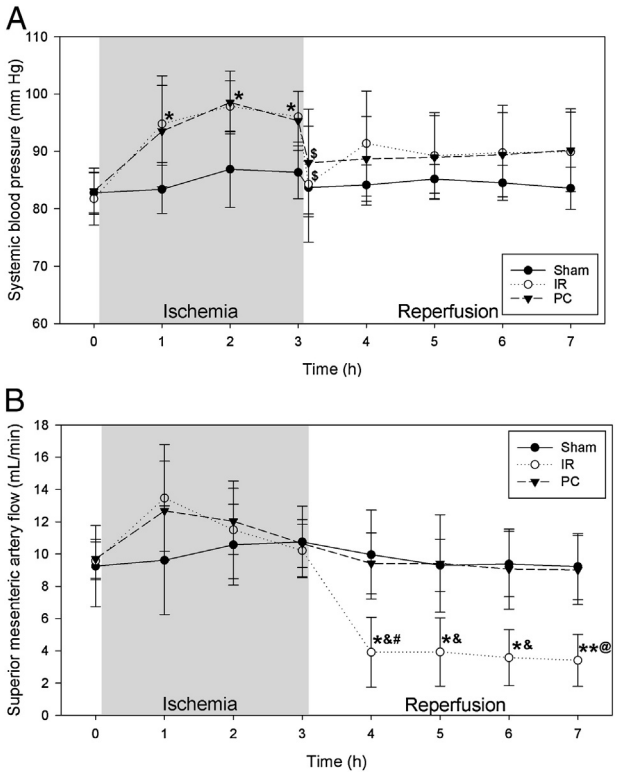

Fig. 1. Macro-hemodynamic measurements. A) *Blood pressure curves*. Blood pressure elevated significantly after aortic occlusion and remained elevated throughout the period of ischemia both in the IR and PC groups compared to the sham operated group. Reperfusion resulted in a significant drop in MAP in both groups (IR and PC) compared to the ischemic values. During reperfusion MAP did not differ significantly from the sham operated group in any measured time points. No significant disparity was detectable in blood pressure values between the IR and the PC groups at any measured time points. B) *Superior mesenteric artery blood flow*. SMA blood flow was significantly impaired during reperfusion in the IR group compared to the sham operated group. PC was able to significantly limit SMA flow impairment during reperfusion at every measured time point compared to the IR group. Values are given as means ± s.e.m. *$P < 0.05$ vs. sham. **$P < 0.01$ vs. sham; &$P < 0.05$ vs. PC; @$P < 0.01$ vs. PC; \$$P < 0.05$ vs. previous measured point; #$P < 0.01$ vs. previous measured point n = 6 per group.

Duodenal microcirculation

Aortic occlusion resulted in a significantly decreased ($P < 0.05$) mucosal flow during the first 2 h of ischemia both in the IR and the PC groups compared to the sham group then normalized by the 3rd hour of ischemia. Reperfusion resulted in a gradual decrease in duodenal microcirculatory flow in the IR group which reached its lowest point by the 2nd hour of reperfusion then it remained constant. PC was able to significantly reduce the changes in microcirculatory flow, in contrast to the IR group in every measured time point after the 1st hour of reperfusion. Sham operated and PC groups did not differ significantly from each other (Fig. 2A).

Microcirculatory flow of jejunal mucosa

As a result of aortic occlusion microcirculatory flow of the jejunal mucosa rose significantly ($P < 0.01$) both in IR and PC groups compared to the sham operated group and remained on a similar level during ischemia. Flow decreased significantly both in the IR ($P < 0.01$) and the PC ($P < 0.05$) groups at the first hour of reperfusion compared to the ischemic values then remained on the same level throughout the measurement period, however PC resulted in significantly ($P < 0.01$) ameliorated microcirculatory flow during reperfusion compared to the IR group (Fig. 2B).

Mucosal microcirculation of the ileum

Microcirculatory flow rose significantly after the occlusion of the infrarenal aorta compared to the sham operated group reaching its maximum after 2 h of ischemia in both IR and PC groups. During reperfusion continuous flow deterioration was detectable in the IR group. Postconditioning was able to maintain ileal microcirculation in a significantly ($P < 0.001$) higher level during reperfusion in contrast to the IR group, however a significantly ($P < 0.05$) reduced mucosal flow still could be observed compared to the sham operated group (Fig. 2C).

Within group comparison of mucosal flow of different bowel sections

No significant difference ($P = 0.879$) could be found among the flow of the different segments in the sham group with two-way repeated measures' ANOVA. In the IR group the mucosal flow of the jejunum and the ileum presented significant ($P < 0.01$) deviation from the duodenal microcirculation during ischemia: while duodenal microcirculatory flow showed impairment during ischemia, microcirculatory flow of ileum and jejunum represented an increase ($P < 0.01$). The flow increment was significantly ($P < 0.01$) larger in the ileum in contrast to the jejunum. During reperfusion mucosal microcirculation showed a gradual decrease in all bowel sections. The smallest diminution in flow was observed in the duodenum; in jejunum, a moderate flow reduction was present while the microcirculation of the ileum impaired principally (Fig. 3). Two-way analysis of variance with repeated measures did not reveal significant differences ($P = 0.189$) among the different bowel sections in the postconditioned group.

Histology

Light microscopic examinations revealed only slight alterations in the sham operated group regarding all three bowel segments. Duodenal mucosa did not show significant alterations either in the IR ($P = 0.093$), or the PC ($P = 0.523$) group compared to the sham operated group. Jejunal sections of the IR group showed significantly higher mucosal damage compared to the sham group ($P = 0.004$). In the PC group, jejunal mucosal injury was significantly reduced ($P = 0.016$) in contrast to the IR group. Similar differences between the experimental groups was seen in case of the ileum (sham vs. IR: $P = 0.003$, IR vs. PC: $P = 0.006$). Within the IR group, ileal sections showed the highest degree

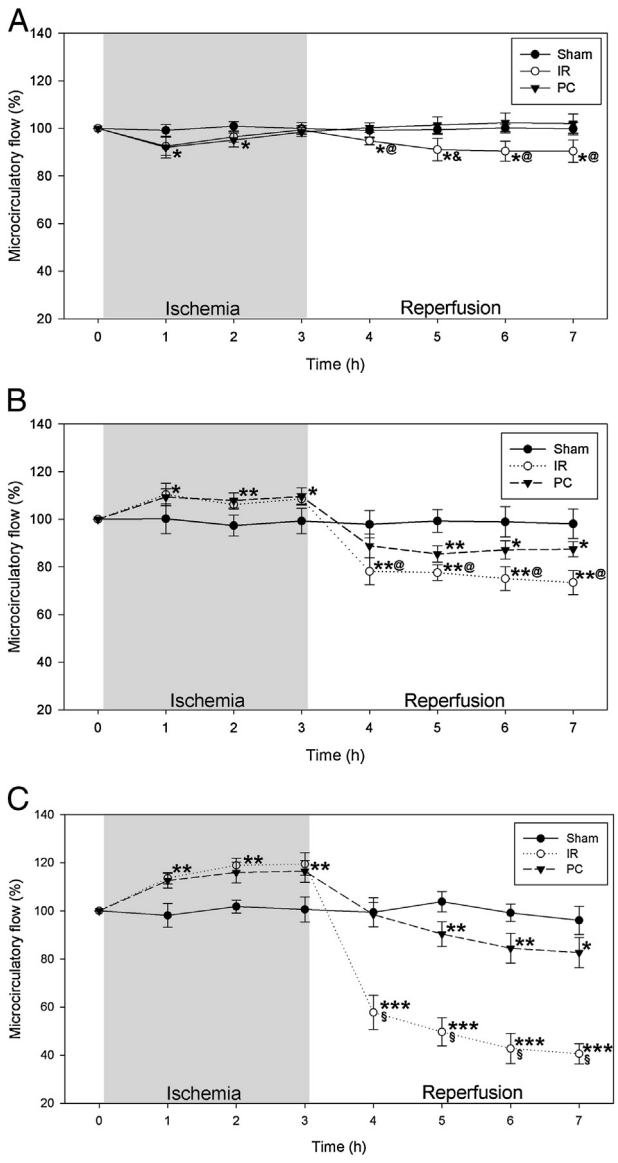

Fig. 2. Microcirculatory flow of small bowel segments. A) *Duodenum*. In the IR and PC groups aortic occlusion resulted in a significant drop in mucosal flow during the first 2 h of ischemia, then flow normalized by the 3rd hour of ischemia. Reperfusion resulted in a gradual decrease in duodenal microcirculatory flow in the IR group. PC significantly improved duodenal microcirculation compared to the IR group. B) *Jejunum*. Microcirculation of the jejunal mucosa rose significantly in both IR and PC groups after aortic occlusion compared to the sham group. Flow decreased both in the IR and the PC groups at the first hour of reperfusion then remained on the same level throughout the measurement period. PC resulted in a significant amelioration of microcirculatory flow during reperfusion compared to the IR group. C) *Ileum*. Microcirculatory flow rose significantly after the occlusion of the infrarenal aorta in both IR and PC groups compared to the sham group. During the reperfusion, continuous flow deterioration was detectable in the IR group. Postconditioning was able to maintain ileal microcirculation in a significantly higher level during reperfusion in contrast to the IR group, however microcirculatory flow did not reach the levels of the sham operated group. Values are given as means ± s.e.m. *P < 0.05 vs. sham; **P < 0.01 vs. sham; ***P < 0.001 vs. sham; &P < 0.05 vs. PC; @P < 0.01 vs. PC; §P < 0.001 vs. PC; n = 6 per group.

of histological injury, while the mildest mucosal damage was found in the duodenum (Fig. 4, Table 1).

Correlation between microcirculation and histological score

Microcirculatory results at the end of the experiment were correlated to the semiquantitative histological score in each animal regardless of group allocation. Significant correlation ($R = -0.911$, $R^2 = 0.847$, $P < 0.001$) was found between the two measured parameters with Spearman's correlation method (Fig. 5).

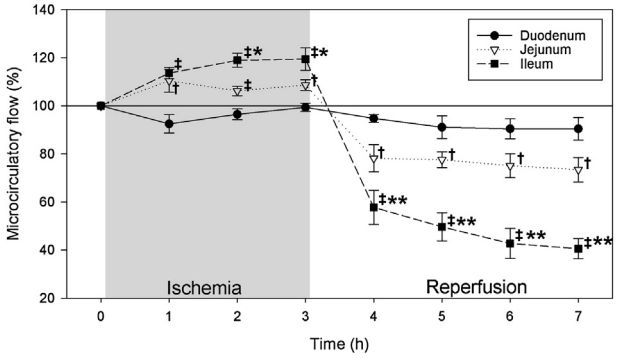

Fig. 3. Comparison of microcirculation in the small bowel sections in the IR group. Jejunal and the ileal flow presented significant deviation from the duodenal microcirculation during ischemia. While duodenal flow showed impairment during ischemia, microcirculatory flow of ileum and jejunum showed a significant increase. The flow increment was significantly larger in the ileum in contrast to the jejunum. During reperfusion mucosal microcirculation showed a gradual decrease in all bowel segments. The smallest diminution in flow was observed in the duodenum; in the jejunum, a moderate flow reduction was present while the microcirculation of the ileum was impaired principally. Values are given as means ± s.e.m. †P < 0.01 vs. duodenum; ‡P < 0.001 vs. duodenum; *P < 0.01 vs. jejunum; **P < 0.001 vs. jejunum; n = 6 per group.

Discussion

It is known for more than two decades that lower limb ischemic–reperfusion injury can lead to gastrointestinal damage (Corson et al., 1992). This observation first occurred in the literature in 1992, since then it has been demonstrated that lower limb ischemia causes an increment in intestinal permeability (Corson et al., 1992; Edrees et al., 2003; Harkin et al., 2001, 2007; Yassin et al., 1997), intestinal pro-inflammatory cytokine expression (Scott et al., 2009), and systemic endotoxin concentration (Cabie et al., 1993; Corson et al., 1992; Foulds et al., 1997; Lau et al., 2000; Yassin et al., 1996, 1998). It has also been demonstrated that lower limb IR can influence the elements of small bowel microcirculation evaluated by intravital videomicroscopy. Accordingly, lower limb IR lead to impaired leukocyte velocity (Wehrens et al., 2002), mesenteric microvascular blood flow (Wehrens et al., 2002), and venular leukocyte recruitment (Scott et al., 2009), as well as to increased leukocyte–endothelial interactions (Sobral do Rosario et al., 1999). The changes in systemic hemodynamics however to this date were not linked to the microcirculatory changes of the small bowel, as well as the flow conditions of the superior mesenteric artery were not assessed before. Furthermore the microcirculatory changes were not previously linked with the degree of the intestinal damage. In this study we aimed to resolve these aforementioned matters.

We found the mean arterial pressure to be significantly elevated after the infrarenal aortic occlusion, in parallel with this the ultrasonic flow probe placed on the SMA registered a rise in blood flow after the placement of the microvascular clip on the aorta. After the initiation of reperfusion a significant drop was detected in SMA flow, while interestingly no systemic blood pressure impairment could be observed.

Previously Wehrens et al. found a similar decline in mesenteric microvascular blood flow after lower limb IR (Wehrens et al., 2002), although in their model a significant drop in blood pressure was also observed. Nevertheless the authors note that “the decrease in mesenteric microvascular blood flow exceeded the decline in blood pressure”.

This phenomenon might be explained by the redistribution of blood flow from the non-vital organs to the ischemic limbs following the release of aortic occlusion, which is most likely caused by splanchnic vasoconstriction (Kologlu et al., 2000) occurring to restore blood pressure to the normal levels after the release of the occlusion. According to our result the state of this circulatory redistribution can last as long as 4 h.

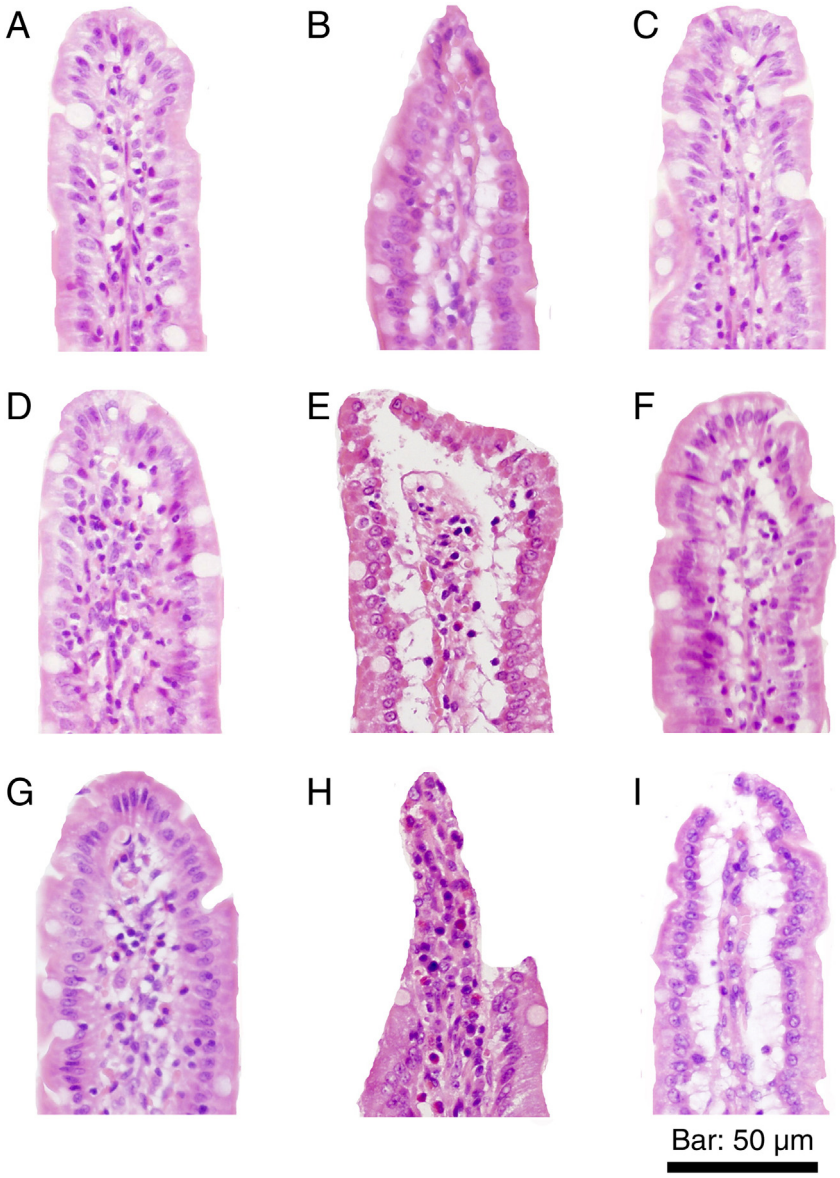

Fig. 4. Representative histological pictures of villi (hematoxylin and eosin staining). Duodenum (A, B, C): light microscopic examinations revealed only slight alterations in the sham operated group (A), which did not reach any significance. Duodenal mucosa did not show significant alterations either in the IR (B), or the PC (C) group compared to the sham operated group. Jejunum (D, E, F): No pathognostic features could be seen in the jejunum in the sham operated group (D). Jejunal sections of the IR group (E) showed higher degree of mucosal damage. In the PC group (F), jejunal mucosal injury was reduced in contrast to the IR group. Ileum (G, H, I): sham operation did not result in any visible changes in the ileum (G). In the IR group severe mucosal injury could be observed in the ileum with denudated villi (H), while in the PC group a more peaceful histological injury could be observed (I). Within the IR group (B, E, H), ileal sections (H) showed the highest degree of histological injury, while the mildest mucosal damage was found in the duodenum (B). Bar: 50 μm.

The changes in macro-hemodynamics, revealed in this study – according to previous findings – might also be represented in small intestinal microcirculatory alterations. Harkin et al. reported earlier that gastric (Harkin et al., 2007), as well as ileal (Harkin et al., 2001) mucosal acidosis – which is considered as an indirect marker of microcirculatory injury (Soong et al., 1997) – increased after bilateral iliac occlusion.

The presence of microcirculatory injury following lower limb ischemia therefore has been indicated so far only by indirect measurements. In our study our objective was to assess microcirculatory changes by means of direct evaluations. Throughout our experiment jejunal and ileal microcirculation showed changes parallel with the alterations of the SMA flow: during ischemia a significantly elevated microvascular flow was present, while after the onset of reperfusion serious flow reduction could be observed in the aforementioned bowel segments. The alterations in ileal microcirculation were more pronounced compared to the jejunum.

Interestingly duodenal microcirculation showed different flow dynamics. During the first 2 h of aortic occlusion duodenal mucosal flow presented a slight impairment, which was resolved by the end of ischemia. During reperfusion duodenal microcirculatory flow decreased, in parallel with the jejunal and ileal microcirculation, although the duodenal microcirculatory impairment was significantly milder than even in case of the jejunum.

Histological damage showed similar alterations to the microcirculatory changes, however in an opposite direction. Accordingly, the highest degree of histological damage was found in the ileum while in the duodenum only mild histological changes were visible. Strong, negative correlation was found between the end reperfusion microcirculatory values and the histological score.

The remote injury to the gut is a serious complication with potentially lethal outcome (Keel and Trentz, 2005), therefore the prevention of the development of these complications are an important clinical claim. Postconditioning is a technique which was reported to be capable

Table 1
Histological scores.

		Histological score
Duodenum	Sham	0 (0; 0)
	IR	1 (0; 1)[#,$]
	PC	0 (0; 1)[$]
Jejunum	Sham	0 (0; 0)
	IR	2 (1; 3)[**]
	PC	1 (0; 1)[†,**]
Ileum	Sham	0 (0; 1)
	IR	3.5 (3; 4)[**,#]
	PC	2 (1; 2)[‡,#]

IR: ischemia–reperfusion, PC: postconditioning.
Legend: Duodenal mucosa did not show significant alteration either in the IR, or the PC group compared to the sham operated group. Jejunal sections of the IR group showed significantly higher mucosal damage compared to the sham group while in the PC group mucosal injury was significantly reduced. The ileal sections revealed similar differences between the experimental groups. Values are expressed as median (25%; 75%). n = 6 per group.
** $P < 0.01$ vs. sham.
† $P < 0.05$ vs. IR.
‡ $P < 0.01$ vs. IR.
# $P < 0.05$ vs. corresponding jejunum.
$ $P < 0.001$ vs. corresponding ileum.

of limiting the degree of skeletal muscle ischemic injury (Charles et al., 2011; Guo et al., 2011; Liang et al., 2013; Mansour et al., 2012; Sinay et al., 2008), a finding which was also confirmed by our results (Turoczi et al., 2014). Furthermore in our previous studies we found that postconditioning is able to limit remote organ damages in the kidneys (Gyurkovics et al., 2011), as well as in the lungs (Garbaisz et al., 2013). In case of remote small intestinal injury Leng et al. demonstrated that postconditioning possesses the ability to limit remote small intestinal injury following lower limb ischemia (Leng et al., 2011), however its effects on mesenterial macro- and microcirculation were not evaluated before.

In our model, although the application of postconditioning did not alter the blood pressure parameters, it was able to significantly improve SMA blood flow, as well as microcirculatory flow in all three small bowel segments. Furthermore our study confirmed the findings of Leng et al., that postconditioning can significantly reduce small intestinal histological damage.

The mechanism behind these remote microcirculation protecting effects of postconditioning is so far unknown in detail. It is known however that postconditioning can improve local microvascular perfusion (Gyurkovics et al., 2011) and reduce leukocyte–endothelial cell interactions through preventing endothelial dysfunction (Widgerow, 2014). These favorable effects on the endothelial cells and on microcirculatory flow might reduce the systemic vasoconstriction. It is also likely that these are not merely local, but systemic effects, therefore it can be assumed that the preservation of the small intestinal flow is a consequence of the positive effect of postconditioning on systemic microcirculation. Neural responses induced by the postconditioning algorithm (Vinten-Johansen and Shi, 2013) might also contribute to the regulation of systemic vasoconstriction and to the preservation of intestinal microcirculation.

From the results of this study the following conclusions can be drawn:

(1) In this study it was discovered that infrarenal aortic occlusion followed by reperfusion results in impairment of SMA blood flow. Parallel to these changes, microcirculatory flow of the three small bowel sections also showed significant deterioration. The microcirculatory flow of the various small bowel segments reacted differently to lower limb ischemia–reperfusion: microcirculation altered mostly in the ileum, while in the duodenum the lowest degree of microcirculatory injury was found, which might be explained by the different collateral blood supply of the various segments from the surrounding arteries (Hebel and Stromberg, 1976).
(2) Nevertheless, from the result of our study it can be anticipated, that the reduction in SMA flow might not be solely responsible for the microcirculatory impairment of the small bowel segments. This assumption is based on the results of the postconditioned group: In contrast to the IR group, PC was able to almost completely abolish the reduction of SMA flow, while in case of the jejunal and ileal microcirculatory flow, postconditioning could not completely limit the microcirculatory damage, whereas in these segments microcirculatory flow did not reach the levels measured in the sham-operated group. This observation suggests that other, so far unidentified factors should be involved in the remote microcirculatory impairment of the small bowel mucosa followed by lower limb ischemia–reperfusion aside from the alterations of the SMA flow.
(3) Histological damage was greater in the bowel sections in which higher degree of microcirculatory impairment was observed. This fact and the strong correlation found between the histological score and the end-experiment microcirculatory values suggest that, regardless of the underlying cause, the microcirculatory changes are directly responsible for the mucosal lesions seen in the histological slides. Thus it can be presumed that remote mucosal injury following infrarenal aortic occlusion is mostly of ischemic origin.

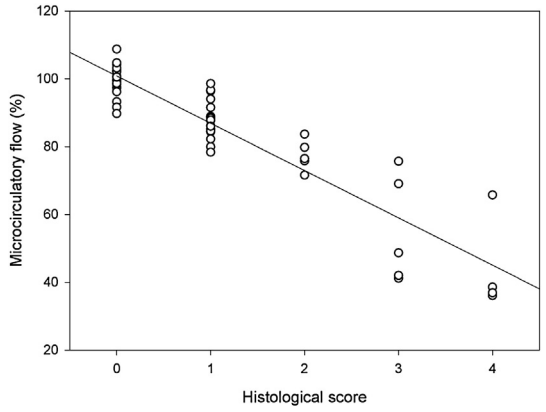

Fig. 5. Correlation between microcirculation and histological score. Microcirculatory results at the end of the experiment were correlated to the semiquantitative histological score in all animals. Significant correlation ($R = -0.911$, $R^2 = 0.847$, $P < 0.001$) was found between the two measured parameters with Spearman's correlation method.

Conclusion

This study demonstrated that revascularization after an infrarenal aortic occlusion resulted in a reduction of superior mesenteric artery flow in rats. The study reports for the first time that mucosal microcirculation of all three small bowel segments showed significant impairment in parallel to the changes of superior mesenteric blood flow with the highest degree in the ileum. Histological alterations mimicked the microcirculatory changes. Postconditioning, through limiting the reduction in SMA blood flow, was able to preserve better microcirculatory flow in the small intestine which has lead to the ameliorated mucosal injury. Important findings of this study are that remote small intestinal damage after lower limb ischemia–reperfusion is directly related to mucosal microcirculatory impairment, and that postconditioning can potently reduce this remote mucosal injury.

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